Enhanced red cell sodium-hydrogen exchange in microvascular angina

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Objectives Enhanced calcium content in arterial smooth muscle cells and altered reactivity of coronary vessels to alkalization have been reported in angina pectoris due to impaired motility of coronary arteries. An altered function of sodium–hydrogen exchange, a ubiquitous membrane transport system that links proton efflux to calcium drifts, may mediate these phenomena.

Design and subjects Twenty patients with microvascular angina (stable effort angina, reversible perfusion defects during effort thallium 201 heart scintigraphy, and angiographically normal coronary arteries) were compared to 20 patients with stable effort angina due to coronary atherosclerosis and 20 healthy subjects. The sodium-hydrogen exchange was defined as the initial fraction of the amiloride-sensitive proton efflux from red cells with inhibited anion exchanger (pHi 600-605) into an Na+-containing medium (pH0 800-805). 12-0-tetradecanoyl-phorbol-13-acetate (TPA, 600 nmol. 1 ') and staurosporine (100 nmol. 1~ ') were used as phosphorylation modulators in vitro.

Results The mean red blood cell Na+/H+ exchange was increased in patients with microvascular angina (451 ±37 vs 142 ±17 and 124±21 umol H+ . 1 cells−1 . min−1, P<001). TPA and staurosporine abolished differences between the groups.

Conclusions Microvascular angina is associated with enhanced Na+/H+ exchange in erythrocytes, probably due to more extensive phosphorylation of the membrane antiporter sites.

Key Words: Microvascular angina, erythrocytes, sodium-hydrogen exchange.

Introduction

Microvascular angina is a common term for exertional angina associated with completely normal coronary angiograms and positive ECG or a thallium scintigraphic response to exercise testing[1]. The disease predominates in middle-aged women and is at least partly responsible for heart damage in hypertension[2] and diabetes mellitus[3]. Myocardial perfusion is impaired in microvascular angina due to enhanced contractility of small myocardial vessels in response to vasopressor agents (e.g. ergonovine)[4] and reduced dilatation in response to vasodilators (such as nitric oxide or dipyridamole)[5]. Abnormal coronary flow reserve[1] is a hallmark of microvascular angina during exercise testing which appears to be almost 100% specific in the diagnosis of microvascular angina[6].

Enhanced contractility of small heart vessels and their reduced dilatation during exercise may involve a system of cell acid/base balance and calcium signalling in endothelial or smooth muscle cells. In this regard, the membrane sodium–hydrogen exchange is of special interest, since this antiporter, ubiquitously expressed in human tissues, promotes exchange of intracellular H+ for extracellular Na+ and links the H+ flux to calcium drift[7].

In the present study, sodium–hydrogen exchange has been assessed in red blood cells of patients with microvascular angina in comparison with patients with coronary atherosclerosis and healthy subjects.

Study population

The composition of the study groups is given below.

Group 1 Microvascular angina

Group 1 comprised 14 women and six men aged 49 ± 2 years (mean ± SE) with effort angina (which had been stable for at least 6 months prior to enrollment), evidence of reversible perfusion defects during effort Thallium 201 heart scintigraphy, and angiographically normal coronary arteries. Mean systolic blood pressure was 131 ± 4 mmHg, mean diastolic blood pressure...
82 ± 4 mmHg, fasting glucose levels were 4.2 ± 0.3 mmol.1⁻¹, mean HbA₁ level was 4.1 ± 0.2%.

Group 2 Coronary atherosclerosis
Group 2 comprised 14 women and six men aged 48 ± 5 years, who had had stable effort angina for at least 6 months prior to enrollment, and angiographic evidence of a more than 50% occlusion of at least one major coronary artery. Mean systolic blood pressure was 126 ± 6 mmHg, mean diastolic blood pressure 84 ± 4 mmHg, fasting glucose levels were 4.0 ± 0.3 mmol.1⁻¹, mean HbA₁ level was 4.2 ± 0.1%.

Group 3 Healthy subjects
Group 3 comprised 14 female and six male volunteers aged 49 ± 2 years, with no history of cardiovascular or other major disorders. Mean systolic blood pressure was 121 ± 8 mmHg, mean diastolic blood pressure 72 ± 7 mmHg, fasting glucose levels were 4.0 ± 0.4 mmol.1⁻¹, mean HbA₁ level was 4.6 ± 0.4%.

Exclusion criteria for all groups were: evidence of valvular disease during routine transthoracic echocardiography, and first-to-second-degree hypertensive relatives. The patients in groups 1 and 3 were treated with aspirin (250 mg . day⁻¹) and isosorbide dinitrate (mean dose 26 ± 4 and 28 ± 6 mg . day⁻¹, respectively; P=0.72). Treatment with calcium antagonists and beta-adrenoblockers was stopped under physician supervision for at least 14 days prior to the examination.

Red blood cell Na⁺/H⁺ exchange measurement
Blood samples, stored with heparin (50 IU . ml⁻¹) and kept ice-cold for no more than 8 h, were taken between 0800 h and 1000 h after the subjects had fasted 8-10 h. After sedimentation (2000 g for 10 min at −2 °C) plasma and white cells were removed and red cells were washed twice with a medium containing 150 mmol. l⁻¹ NaCl and 5 mmol. l⁻¹ sodium phosphate buffer (pH 7.4).

Na⁺/H⁺ exchange
Sodium–hydrogen exchange was defined as the amiloride-inhibited fraction of H⁺ efflux from red cells into a Na⁺-containing medium, as described by Escobales and Canessa[8] and Orlov et al.[9]. One hundred microlitres of packed red cells were placed into 1.9 ml of medium consisting of 150 mmol. l⁻¹ NaCl, 1 mmol. l⁻¹ KCl, 1 mmol. l⁻¹ MgCl₂ and 10 mmol. l⁻¹ glucose, and incubated for 5 min at 37 °C. The pH value of the cell suspension was adjusted by the slow addition of 0.2 HCl solution in 150 mmol. l⁻¹ choline–chloride. The anion exchanger was inhibited by 200 μmol. l⁻¹ 4,4'-disothiocyanostilbene-2,2'-disulphonic acid (DIDS), and the medium pH was adjusted rapidly to 7.95-8.05 by the addition of 0.05 N NaOH solution in 150 mmol. l⁻¹ choline–chloride. Each experiment was redone with 500 μmol. l⁻¹ amiloride (50 μmol. l⁻¹, a half-inhibitory effect was seen) added before DIDS. The kinetics of the proton efflux were registered by means of a 91-15 electrode (Orion, Cambridge, MA, U.S.A.) connected to a PHM-64 (Radiometer, Copenhagen, Denmark).

The maximal initial velocity (Vₘₐₓ) of the exchanger was determined as (∆pH₁.− ∆pH₂) x b. m⁻¹. t⁻¹, where ∆pH₁ and ∆pH₂ are the initial rates of medium acidification in the absence and presence of amiloride, respectively; b is the buffer capacity of the incubation medium (determined by titration of 1.9 ml medium with NaOH and HCl from pH 6.0 to 8.0); m is the red cell volume; t is the incubation time.

In separate experiments, 12-0-tetradecanoyl-13-acetate (TPA, 600 nmol. l⁻¹) and staurosporine (100 nmol. l⁻¹) were used as phosphorylation modulators[10].

Statistical analysis
All values were counted as mean ± SE. Significance was calculated by the two-tailed Student’s t-test. Correlation between the values was by the Pearson Product Moment method. A P value less than 0.05 was considered statistically significant.

Results
The mean sodium–hydrogen exchange in the red cells of patients with microvascular angina (Group 1) was enhanced three-fold compared to patients with coronary atherosclerosis (Group 2) and healthy subjects (Group 3, Fig. 1), although there was a wide distribution of individual values in Group 1.

There was no correlation between sodium–hydrogen exchange and age, sex, symptom duration, blood pressure or glucose balance in any of the groups. TPA added to the incubation medium enhanced the sodium–hydrogen exchange activity, whereas staurosporine inhibited it (Fig. 2). Both phosphorylation modulators abolished the initial difference in sodium–hydrogen exchange levels between the groups.

Discussion
Enhanced calcium content in arterial smooth muscle cells and altered reactivity of coronary vessels to pressor (ergonovine) and depressor (dipiridamol) agents have been revealed in vasospastic angina, together with impaired reactivity of the coronary vessels to alkalining factors (e.g. hyperventilation and a tris-buffer infusion)[11]. The membrane H⁺ fluxes and cell...
calcium-dependent processes are coupled by several membrane mechanisms, one of which— the Na⁺/H⁺ exchange— has been found enhanced three-fold in patients with microvascular angina in the present study (Fig. 1). The increased exchange of intracellular H⁺ for extracellular Na⁺ during exercise-induced acidosis may trigger Na⁺/Ca²⁺ exchange and voltage-dependent membrane Ca²⁺ channels and cause intracellular calcium overload that underlies enhanced cell contractility and delayed or diminished dilatation in response to circulating or paracrine stimuli.

The amiloride (200 μmol. l⁻¹) sensitive Na⁺/H⁺ exchanger represents an integral protein of plasma membranes, which is ubiquitously expressed in human cells, including blood and muscle cells. Its activity in red blood cells correlates well with the exchanger velocity in smooth muscle cells[7,9]. Sodium–hydrogen exchange measurements in erythrocytes are even more accurate, since red cells lack genomic and rapid posttranscriptional changes in protein synthesis, and a large number of intracellular compartments responsible for interferences between the intra- and extracellular fluxes of Na⁺ and H⁺ in other cells.

Sodium–hydrogen exchange enhancement in microvascular angina may be due to excessive methylation, carboxymethylation and phosphorylation of an equal or a higher number of cell exchangers. To distinguish between these options, we used a phosphorylation inhibitor (staurosporine) and stimulator (12-O-tetradecanoyl-13-acetate, TPA) in the sodium–hydrogen exchange study in vitro. Since staurosporine completely inhibited the sodium–hydrogen exchange in erythrocytes of microvascular angina patients (Fig. 2), phosphorylation of the exchangers was concluded to be the main mechanism of sodium–hydrogen exchange enhancement in microvascular angina. TPA stimulated the sodium–hydrogen exchange in all studied groups, and the maximally TPA-activated sodium–hydrogen exchange was similar in all the groups (Fig. 2). Since maximally TPA-activated sodium–hydrogen exchange corresponds to a state with maximally phosphorylated exchanger sites, the total number of Na⁺/H⁺ exchangers was therefore equal in the three groups. We concluded that the sodium–hydrogen exchange enhancement in microvascular angina was due to more extensive phosphorylation of the same membrane exchangers rather than to possible exchanger synthesis de novo.

The same pattern of sodium–hydrogen exchange stimulation has been reported in essential
hypertension\cite{10,12} and diabetes mellitus\cite{13} where it is thought to be a result of the genetically determined hyperactive state of protein kinase C\cite{13,14}. Interestingly, both essential hypertension and diabetes mellitus are associated with microvascular angina\cite{2,3}. In the present study, a special effort was made to exclude those with a tendency to hypertension or hyperglycaemia. Therefore, the question remains whether the enhanced sodium–hydrogen exchange in essential hypertension and diabetes can also be related to the subsets of patients who are prone to impaired contractility in small heart vessels.

On the other hand, the three conditions — microvascular angina, essential hypertension and non-insulin dependent diabetes mellitus — represent the well known states associated with hyperinsulinaemia\cite{15}. Hyperinsulinaemia might also account for the sodium–hydrogen exchange increase, since insulin is found to augment sodium–hydrogen exchange in vitro via tyrosine kinase and protein kinase C pathways (W.K., data not shown).

Further in-depth investigations, both at the population level, and aimed at biochemical measurement of endogenous phosphorylation, are needed to confirm our assumptions. However, even the preliminary results presented in this study strongly suggest the potential role of sodium–hydrogen exchange as a marker of impaired contractility of coronary vessels.

References

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